International Journal of Medicine
and Pharmaceutical Science (IJMPS)
ISSN (P): 2250-0049; ISSN (E): 2321-0095
Vol. 8, Issue 2, Feb 2018, 11-18


TRANS STELLAR
•Journal Publications •Research Consultancy

Original Article

# ANALYSIS OF DRUG CONTENTS OF CEFOTAXIME FORMULATIONS AVAILABLE IN THE LOCAL MARKET AND THE EFFECT OF STORAGE CONDITIONS

**HADIER A. THAMER[1], NIZAR S. HADDAD[2] & ABDULLAH M. JAWAD[3]**

[1]*Bsc (Pharm), Msc Student (Pharmacology), Department of Pharmacy, Al-Sader Teaching Hospital, Basrah, Iraq*

[2]*MBCHB, CAMB, Assistant Professor in Chemical Pathology, College of Medicine, University of Basrah, Iraq*

[3]*MBCHB Ph. D, Professor of Pharmacology, College of Medicine, University of Basrah, Iraq*

***ABSTRACT***

***Background:** Low quality antimicrobial drugs represent a world-wide problem. Beta-lactams are the main types of antimicrobials found to be substandard or counterfeit. Environmental conditions can play an important role in maintaining drug safety and quality .*

***Aim:** To investigate whether the pharmaceutical formulations of cefotaxime, available in the local drug market, contain appropriate amounts of the active ingredients, and how their contents are changed after being exposed to different storage conditions.*

***Methods:** Five brand names of cefotaxime vials available in authorized pharmacies in the local drug market, are selected. Drug contents were measured by HPLC (Agilent, UK) using C18 column. Other tests performed include mass uniformity (weight) test, pH of reconstituted solution, identification of impurities, and color of solid dosage forms. Samples were re-analyzed after being exposed to different temperature and humidity levels for 30 days .*

***Results:** No substandard and/or counterfeit drugs were found among the tested samples of cefotaxime. The amount of drugs of all tested samples was more than 90% of the labeled one. Tested samples of the drug were stable when stored at a temperature of 35 C˚ for 30 days. When they were stored at temperature of 50 C˚ for 30 days, part of the active ingredients was lost which reached up to 7.4%, with variation between the 5 brands tested. This caused the active ingredients in two brands to fall below the acceptable 90% of the labeled amount. The effect of humidity on the stability of collected samples of different packaged brands of cefotaxime was insignificant .*

***Conclusion:** The finding that none of the 5 brands of cefotaxime are of low quality is reassuring and can give the treating physician the choice among different brands of these drugs according to price and availability. Care should be taken in storing or transporting these drugs at high (50 C˚) temperature.*

***KEYWORDS:** Low Quality Antimicrobials, Counterfeit Medicines, Beta-Lactam Antibiotics, HPLC & Cefotaxime*

**Received:** Jan 22, 2018**; Accepted:** Feb 12, 2018**; Published:** Mar 03, 2018**; Paper Id.:** IJMPSAPR20183

## INTRODUCTION

Low quality medicines such as substandard or counterfeit medicines become a global problem with increasing rate in the last decade [1]. According to both the United States Food and Drug Administration (US-FDA) and the World Health Organization (WHO), up to 10% of drugs worldwide are low quality medicines [2]. In developing countries, counterfeit medicines account for 10 to 30% of all medicines used, with rates higher than

30% in some regions of Latin America, South-East Asia or Sub- Saharan Africa [3]. Substandard and counterfeit medicines have serious effects on both patients and public health. They might also affect the economy of countries [4]. One of the serious effects on patients is that they can increase the morbidity and mortality of patients due to lack or insufficient therapeutic active ingredients [2]. Spread of internet pharmacies is a recent factor that has increased counterfeit medicines since the internet pharmacies, in most countries, have no regulations or registration processes [5]. Fifty percent of the counterfeit or substandard medicines involve antimicrobial drugs, and 78% of incidents occurred in poor countries [6]. Antibiotics are, therefore, the most commonly falsified medicines [7]. Substandard antimicrobial drugs can result in administration of sub-optimal doses, which can lead to the development of antimicrobial resistance, especially with the use of a combination of antimicrobials such as anti-tuberculosis and antimalarial drugs [2].

Many methods used for the detection of substandard/counterfeit antimicrobial medications including inspection, dissolution test, colorimetric techniques, chromatography techniques and mass spectrometry, These methods could be simple as inspection of the physical and chemical characteristics of the drug to more complex methods such as high-performance liquid chromatography (HPLC) [6]. HPLC can be used to measure active ingredients in a sample qualitatively and quantitatively, and also to identify impurities [2].

## METHODS

Five brand products of cefotaxime were purchased from authorized local drug stores in the center of Basrah city during the last three months of 2015. The samples should have the same batch number. Drug contents of the active ingredient were measured by high performance liquid chromatography (HPLC, Agilent, UK) using C18 column using mobile phase that consisted of 150 ml of acetonitrile added to 850 ml of 10mM of ammonium acetate buffer solution (pH 5.5) with shaking, filtered through a 0.45 µm filter paper and degassed for 15 minutes in ultrasonic bath. Other tests performed include mass uniformity test (weight), pH of reconstituted solution, and identification of impurities which were performed according to USP pharmacopeia, 2011[8]. Samples were re-analyzed and assessed after being exposed to different environmental conditions (Table 1).

**Table 1: Stress Stability Studies and Parameters Measured**

| Groups | Storage Conditions | Storage Time | Time for Measurement | Parameters Measured |
|---|---|---|---|---|
| Group A | 35 C˚ | 30 days | 0, 14, 30 days | - Weight<br>- Color<br>- pH<br>- Activeingredient<br>- Impurities |
| Group B | 35 C˚ with 75% RH* | 30 days | 0, 14, 30 days | |
| Group C | 35 C˚ | 30 days | 0, 14, 30 days | |
| Group D | 35 C˚ with 75% RH* | 30 days | 0, 14, 30 days | |

*RH = Relative Humidity

Samples were placed in hot air oven with digital temperature controller (Memmert Universal Oven UN110) that can keep the temperature as required during the period of stress (30 days).

Humidity control was performed using saturated with pure sodium chloride solution in closed spaces. The saturated solution of sodium chloride can provide constant 75% relative humidity with less than 5% variation which can be measured by humidity sensor [9].

A stock solution of cefotaxime was prepared in water (HPLC grade). Working solutions were, then, prepared by diluting aliquots of stock solutions with the mobile phase. 100 µl of each working solution were injected into the chromatograph and the retention time, area of major peaks and peak height were recorded.

The amount of cefotaxime sodium in pharmaceutical formulations was determined by accurately-weighing the contents of 10 vial of cefotaxime injections from each of the five brand samples. The average weight of each vial content was taken and dissolved in 10 ml of distilled water (that was included within the vial cefotaxime package). From this, a working sample solution of 55 µg/ml was prepared using the mobile phase in 10 ml volumetric flasks and filtered through a 0.45 µm filter paper, then degassed for 15 min in ultrasonic bath. 100 µl of working sample solution were, then, injected into the chromatograph and the response was recorded. The test was repeated after 14 and 30 days of incubation in the oven.

## RESULTS

### Analysis of Cefotaxime Vial Before and After Stress Stability Test Weight Variation of Cefotaxime Vial Powder after Storage for One Month at 35 c˚ and 50 c˚ with Ambient or 75% Relative Humidity

The powder of the 5 brands of cefotaxime vials did not show a significant weight variation after one month storage under 35 and 50 C˚ with ambient or 75% relative humidity (Table 2).

**Table 2: Weight Variation of Cefotaxime Vial Powder after Storage for One Month at 35 and 50 C˚ with Ambient or 75% Relative Humidity**

| Cefotaxime 1000 mg Brand Products | Average Weight in mg (n=10 vials) | Average Weight in mg After Incubation for One Month (n= 3 vials): Group A Group B Group C Group D | | | |
|---|---|---|---|---|---|
| | | 35 C˚ | 35 C˚with 75% Humidity | 50 C˚ | 50 C˚with 75% Humidity |
| Cefotaxime LG | 1070 ± 36 | 1068 ± 12 | 1074 ± 14 | 1067 ± 23 | 1062 ± 11 |
| Loraxime 1000 | 1040 ± 23 | 1049 ± 20 | 1047 ± 22 | 1035 ± 16 | 1036 ± 07 |
| Cefotaxime LDP | 1044 ± 26 | 1052 ± 09 | 1037 ± 19 | 1032 ± 08 | 1051 ± 18 |
| Kon-SEFATAX | 1098 ± 21 | 1088 ± 15 | 1094 ± 13 | 1086 ± 10 | 1082 ± 21 |
| Fortax | 1050 ± 31 | 1062 ± 10 | 1049 ± 15 | 1040 ± 19 | 1048± 13 |

Data are presented as mean of ten vial weight for each sample ±SD

### pH of Cefotaxime Solution in Distilled Water after Storage for One Month at 35 and 50 C˚ with Ambient or 75% Relative Humidity

The slight decrement in pH values after different storage conditions is statistically insignificant as described in table 3.

**Table 3: Ph Variation of Cefotaxime Vial Reconstitution in Distilled Water after Storage for One Month at 35 and 50 C˚ with Ambient or 75% Relative Humidity**

| Brands of Cefotaxime 1000mg Vials Reconstituted in Distilled Water | pH (n=10 vials) | pH after Incubation for One Month (n= 3 vials): Group A | Group B | Group C | Group D |
|---|---|---|---|---|---|
| Cefotaxime LG | 6 ±0.2 | 6 ± 0.1 | 5.9 ± 0.2 | 5.9 ± 0.3 | 5.8 ± 0.1 |
| Loraxime 1000 | 6 ± 0.4 | 5.9 ± | 5.9 ± 0.3 | 5.8 ± 0.2 | 5.8 ± 0.1 |
| Cefotaxime LDP | 5.9 ± 0.3 | 5.9 ± | 5.9 ± 0.2 | 5.8 ± 0.3 | 5.8 ± 0.2 |
| Kon-SEFATAX | 6.1 ± 0.2 | 6.0 ± | 5.9 ± 0.1 | 5.9 ± 0.1 | 5.8 ± 0.2 |
| Fortax | 5.9 ± 0.4 | 5.9 ± | 5.9± 0.1 | 5.8 ± 0.3 | 5.8 ± 0.1 |

Data are presented as meanof ten vial pH analyses for each sample ± SD

**Identification of the Active Ingredients (Cefotaxime) by Near Infrared Spectrophotometry**

All samples are identified by near infrared spectrophotometry and found to contain the active ingredient cefotaxime (Figure 1).

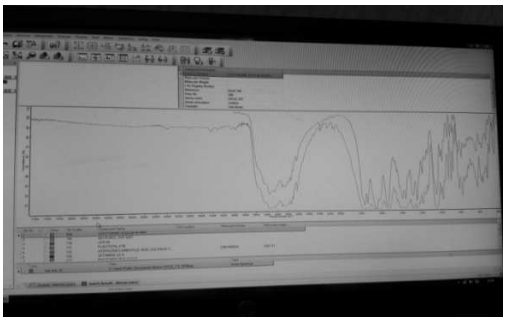

**Figure1: Near Infrared Spectrophotometry of Cefotaxime**

**Assay of Cefotaxime in Vial Samples stored Under Different Conditions Using High Performance Liquid Chromatography (HPLC)**

**Assay of Cefotaxime Amount in Vial Samples before Stress Stabilities Studies**

The amount of cefotaxime in the 5 brand products of cefotaxime vials measured by HPLC was found to range between 91.4% and 98.9% of the labeled amount which is 1000 mg (Table 4, Figure 2).

**Table 4: Assay of Cefotaxime Amount in Vial Samples of Cefotaxime From Different Brands Using HPLC Method**

| Cefotaxime Samples | Amount of Cefotaxime No. of Vial = 10 | | Percent of Labeled Amount |
|---|---|---|---|
| | Labeled | Measured | |
| Cefotaxime LG | 1000 mg | 982 mg ± 2.4 mg | 98.2% |
| Loraxime 1000 | 1000 mg | 914 mg ± 6.5 mg | 91.4% |
| Cefotaxime LDP | 1000 mg | 989 mg ± 3.3 mg | 98.9% |
| Kon-SEFATAX | 1000 mg | 916 mg ± 6.4 mg | 91.6% |
| Fortax | 1000 mg | 921 mg ± 3.2 mg | 92.1% |

Data are presented as means often via l HPLC analysis ± SD with percent change from labeled amount.

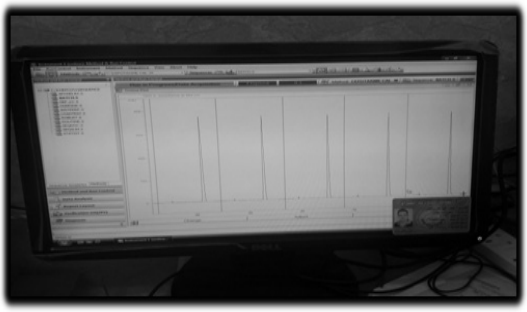

**Figure 2: Five Replicate Injections of 55ug/ml of Cefotaxime**

**Assay of Cefotaxime Amount after 14 and 30 day-Exposure to 35 C˚ with Ambient or 75% Relative Humidity**

The changes in cefotaxime amount in vials after storage for 14 and 30 days at ambient or 75% relative humidity with a temperature of 35 C˚ are insignificant mounting up to 0.8% of the pre-exposure measurements after 30 days storage

(Table 5 and table 6).

**Table 5: Amount of Cefotaxime in Vials Measured after 14 and 30 Day-Exposure to 35 C˚ and Ambient Humidity Between 35% to 45% Using HPLC Method**

| Samples | Amount (mg) Before Exposure | 14 days After Exposure | | 30 days After Exposure | |
|---|---|---|---|---|---|
| | | Amount (mg) | % Change | Amount (mg) | % Change |
| Cefotaxime LG | 982 ± 2.4 | 980 ± 1.9 | - 0.2% | 977 ± 3.9 | - 0.5% |
| Loraxime 1000 | 914 ± 6.5 | 909 ± 7.2 | - 0.6% | 907 ± 3.0 | - 0.5% |
| Cefotaxime LDP | 989 ± 3.3 | 987 ± 4.5 | - 0.2% | 984 ± 2.2 | - 0.5% |
| Kon-SEFATAX | 916 ± 6.4 | 914 ± 4.8 | - 0.2% | 912 ± 1.2 | - 0.4% |
| Fortax | 921 ± 3.2 | 918 ± 5.7 | - 0.3% | 914 ± 4.9 | - 0.8% |

Data are presented as means of three replicate HPLC analysis ± SD with percent of change from pre-exposure amount

**Table 6: Amount of Cefotaxime in Vials after 14 and 30 Day-Exposure to 35 C˚ and 75% Relative Humidity**

| Samples | Amount (mg) Before Exposure | 14 days After Exposure | | 30 days After Exposure | |
|---|---|---|---|---|---|
| | | Amount (mg) | % Change | Amount (mg) | % Change |
| Cefotaxime LG | 982 ± 2.4 | 979.5± 1.3 | - 0.3% | 975± 0.3 | - 0.7% |
| Loraxime 1000 | 914 ± 6.5 | 911± 2.5 | - 0.3% | 907± 0.9 | - 0.8% |
| Cefotaxime DP | 989 ± 3.3 | 987.4± 5.0 | - 0.2% | 986± 2.2 | - 0.3% |
| Kon-SEFATAX | 916 ± 6.4 | 914± 2.2 | - 0.2% | 912± 3.4 | - 0.4% |
| Fortax | 921 ± 3.2 | 919± 1.8 | - 0.2% | 917± 4.4 | - 0.4% |

Data are presented as means of three replicate HPLC analysis ± SD with percent of change from pre-exposure amount

**Assay of Cefotaxime Amount, 14 and 30 Days after Exposure to 50 C˚ with Ambient or 75% Relative Humidity**

The loss in cefotaxime amount after storing the vials under 50 C˚ with ambient or 75% relative humidity is more than when the storage conditions under 35 C˚, ranging from 0.87% to 1.8% at 14 days, increased to 1.7% to 4.6% after 30 days. The amount which was lost under 50C˚ and ambient humidity is not increased when relative humidity increased to 75% as describe in table 7 and table 8.

**Table 7: Cefotaxime Amount Measured after 14 and 30 day-Exposure to 50 C˚ with Ambient Humidity**

| Samples (n = 3 Vials of Each Brand Product) | Amount (mg) Before Exposure | 14 days after Exposure | | 30 days after Exposure | |
|---|---|---|---|---|---|
| | | Amount (mg) | % Change | Amount (mg) | % Change |
| Cefotaxime LG 1000 | 982± 2.4 | 964± 2.5 | - 1.8% | 952± 3.1 | - 3.1% |
| Loraxime 1000 | 914± 6.5 | 903± 4.1 | - 1.2% | 889± 6.1 | - 2.7% |
| Cefotaxime LDP 1000 | 989± 3.3 | 980± 2.2 | - 0.9% | 968± 3.7 | - 2.1% |
| Kon-SEFATAX | 916± 6.4 | 908± 1.2 | - 0.87% | 873± 6.4 | - 4.6% |
| Fortax | 921± 3.2 | 913± 4.4 | - 0.88% | 905± 2.5 | - 1.7% |

Data are presented as means three replicate HPLC analysis ± SD with percent change from pre-exposure amount

**Table 8: Cefotaxime Amount Measured after 14 and 30 Day-Exposure to 50 C˚with 75% Relative Umidity**

| Samples | Amount (mg) Before Exposure | 14 days After Exposure | | 30 days After Exposure | |
|---|---|---|---|---|---|
| | | Amount (mg) | % Change | Amount (mg) | % Change |
| Cefotaxime LG | 982 ± 2.4 | 968± 2.5 | 1.42% | 958.8± 6.4 | 2.36% |
| Loraxime 1000 | 914 ± 6.5 | 904± 3.2 | 1.09% | 893 ± 5.5 | 2.29% |

| | | | | | |
|---|---|---|---|---|---|
| Cefotaxime LDP | 989 ± 3.3 | 983 ±1.4 | 0.70% | 970 ± 1.6 | 1.92% |
| Kon-SEFATAX | 916 ± 6.4 | 908± 3.8 | 0.87% | 879 ± 6.3 | 4.03% |
| Fortax | 921 ± 3.2 | 914 ± 7.2 | 0.76% | 904 ± 2.9 | 1.85% |

Data are presented as means ± SD with percent change from pre-exposure amount

## DISCUSSIONS

Research on the prevalence of low-quality medicines increases in recent years in response to increased use of these type of medicines. This occurs, especially in developing countries where there is a weakness in regulating the pharmaceutical, manufacturing and distribution processes, and defects in controlling importing activities [10].

Antimicrobial drugs are a major group of drugs affected by the problem of low quality medicines due to their high demands to treat infection, high price, with low availability of authentic antimicrobials. This is, in addition, to the fact that most of the antimicrobials are heat sensitive compounds [2].

The main types of substandard antibiotics are those with fewer amounts of active ingredients, which may be due to defects in the manufacturing process or due to degradation by stressful environmental conditions. Counterfeit medicines usually appear with no active ingredients [11].

It might be hard to differentiate between substandard and counterfeit medicines, and many authors use the term (low or poor quality medicines) to refer to both [12].

Most studies on substandard/counterfeit antimicrobial drugs investigated beta-lactam group and focused on old, commonly used drugs such as amoxicillin (capsules), which is the most frequently used antimicrobial drugs in developing countries [13].

There are relatively few studies on third generation cephalosporins as they are less used when compared to old cephalosporins and penicillins. Only few cases of substandard cefotaxime vials had been reported, for example in Russia in 2004[2], Substandard ceftriaxone vials had also been reported [14].

In the present study, 5 brand products of cefotaxime 1gram vials representing parenteral third generation cephalosporins in the local drug market were collected. The extent to which these cefotaxime brand products meet pharmacopeia standard criteria in terms of uniformity of content, uniformity of weight, physiochemical properties and the stability in the stress studies to simulate environmental conditions of storage and transport, had been investigated. The present study, also, tried to find out whether any of these cefotaxime brand formulations are counterfeit (e.g. containing no active ingredients) or not. However, Delepierre et al [7], found in their review that oral dosage forms are more counterfeited by 4.5 folds than injections.

The ability of simple methods as color of the dosage form, pH of their reconstituted solution in water and weight variation test, to detect substandard/ counterfeit cefotaxime, is limited.

The present study showed that none of the tested five brand products of cefotaxime vials available in the local market was low in quality in terms of their contents of the active ingredient (cefotaxime, as measured by HPLC), presence of impurities, uniformity of weight, pH of their reconstituted solution and color changes. These findings are reassuring and give an idea that at least for the samples tested, no low/poor quality cefotaxime was found.

The tested brand products varied in their contents of the active ingredients, but none of them contained less than 90% of the labeled amount. Humidity as high as 75% did not seem to affect the quality of the un-opened dosage forms of cefotaxime. Temperature, on the other hand, affected the quality by decreasing the amount of the active ingredients and increasing degradation products, particularly when the dosage forms of cefotaxime were stored at 50 C˚ for one month. The summer season is hard in Basrah, and the temperature can reach 50 C˚ or more under the shade which may continue for around one month. The dosage forms were exposed, in the present study, to similar conditions. They were found to cause changes in the amount of active ingredient, which had reached up to 4.6% and the amount of active ingredients in some brand products reached to below 90% of the labeled amount. Differences in the percentage of degradation between the five brands were not found to be related to the expiry dates labeled on the brand samples.

Up to our knowledge, there is no study on the quality of antimicrobial drugs in the Iraqi drug market to compare with. Iraq applies drug rules and regulations different from other countries in the region, having different resources and rates of individual incomes, which make comparison difficult [2,12].

Many developed countries (e.g. USA, European Union, Japan) depends on performing stability studies on the guidelines of The International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). Although these guidelines are not designed for registration of medicines exported to other parts of the world, they classify countries according to their environmental conditions (Iraq is described as dry hot/humid climate zone). The guidelines did not set criteria specific to our region or to the transport conditions to those areas [8].

## CONCLUSIONS

In conclusion, and unexpectedly, no substandard and/or counterfeit drugs were found among the tested samples of cefotaxime collected from authorized sources in the local drug market in Basrah. However, an eye should be kept on storing or transporting these drugs at a temperature of 50 C˚ or more since part of the active ingredient is lost. Relative humidity up to 75% seems to have no significant effect on packaged cefotaxime formulations.

***REFERENCES***

1. *World Health Organization. Definitions of SSFFC Medical Products, 2016. Available at: http://www.who.int/medicines/regulation/ssffc/definitions/en/ (Accessed: June 1, 2016).*
2. *Kelesidis T, Falagas ME. Substandard/counterfeit antimicrobial drugs. Clinical Microbiology Reviews 2015; 28: 443-446.*
3. *United Nations Office on Drugs and Crime. Transnational Organized Crime Threat Assessment. United Nations Office on Drugs and Crime, UNODOC, Vienna, 2010.*
4. *Deisingh AK. Pharmaceutical counterfeiting. Analyst 2005; 130: 271-279.*
5. *Jackson, G., Patel, S. and Khan, S. Assessing the problem of counterfeit medications in the United Kingdom. Int J ClinPract2012; 66: 241-250.*
6. *Kelesidis, T., Kelesidis, I., Rafailidis, P. I. and Falagas, M. E. Counterfeit or substandard antimicrobial drugs: a review of the scientific evidence. J AntimicrobChemother 2007; 60:214-236.*
7. *Delepierre, A., Gayot, A. and Carpentier, A. Update on counterfeit antibiotics worldwide; public health risks. Med Mal Infect 2012; 42: 247-255.*

8. *Guidelines, International Conference on Harmonization. Validation of analytical procedures: text and methodology 2005; Q2 (R1): 1.*

9. *Young JF. Humidity control in the laboratory using salt solutions-a review. Journal of Applied Chemistry 2007 4; 17(9):241–245.*

10. *Khan, A. Y. and Ghilzai, N. M. K. Counterfeit and substandard quality of drugs: the need for an effective and stringent regulatory control in India and other developing countries. Indian J Pharmacol2007; 39: 206.*

11. *Bate R., Mooney, L., Hess, K., Milligan, J. and Attaran, A. Anti-infective medicine quality: analysis of basic product quality by approval status and country of manufacture. Res Rep Trop Med 2012; 3: 57-61.*

12. *Almuzaini T, Choonara I, Sammons H. Substandard and counterfeit medicines: A systematic review of the literature. BMJ Open. 2013 Aug;3(8):e002923.*

13. *Kyriacos, S., Mroueh, M., Chahine, R. P. and Khouzam, O. Quality of amoxicillin formulations in some Arab countries. J Clin Pharm Ther 2008; 33: 375-379.*

14. *Yoshida, N., et al. A cross-sectional investigation of the quality of selected medicines in Cambodia in 2010. BMC PharmacolToxicol 2014; 15: 1.*

15. *Okorie, O., Abayomi, E. and Onyinyechi, E. Pharmaceutical quality analysis of Ceftriaxone sodium brands marketed in southern Nigeria. Br J Pharm Res 2016; 9: 1–8.*